THE GENERAL BIOLOGICAL SIGNIFICANCE OF CHANGES IN THE PERMEABILITY OF THE SURFACE LAYER OR PLASMA-MEMBRANE OF LIVING CELLS.[1]

RALPH S. LILLIE.

The osmotic properties of living cells have been the subject of repeated investigation since the fundamental experiments of Pfeffer and de Vries, and especially since the rise of the theory of osmotic pressure and the extension of the gas laws to dissolved substances by van't Hoff in 1887.[2] One remarkable result, of the highest significance for general physiology, has been perhaps the main outcome of these researches; living cells of the most diverse kinds have been found to possess in common a high degree of physical impermeability to most of the diffusible non-colloid substances normally occurring in the cells and in their surroundings. The surface film or so called plasma-membrane of cells, while readily permeable to water, offers a highly efficient barrier — at least during the resting or unstimulated state — to the entrance or exit of many dissolved substances of relatively low molecular weight, even of those normally present in protoplasm, as sugars, amino-acids, and neutral salts. This impermeability is a property of the normal living cell, unmodified by experimental conditions. A direct proof of this is the characteristic turgor of plant cells, which is due to the osmotic pressure of crystalloid substances dissolved in the cell-sap; it is clear that the osmotically active substances — sugars, organic acids, and other relatively simple compounds — are ordinarily unable to traverse the limiting surfaces of the protoplasm; were they able to do so the maintenance of the high internal pressures known to exist would be impossible. Such impermeability is a peculiarity of the *living*

[1] From the Physiological Laboratory, Department of Zoölogy, University of Pennsylvania.

[2] A full account of the earlier researches will be found in Hamburger's "Osmotischer Druck und Ionenlehre in den medizinischen Wissenschaften," Wiesbaden, I. F. Bergmann, 1902–4. See also Höber's "Physikalische Chemie der Zelle und der Gewebe, 2d ed., Leipzig, Engelmann, 1906.

cell and disappears at death, which is always associated with a loss of turgor and a diffusion of dissolved materials (coloring matters, etc.) from the cell. The increase in permeability may be the consequence, or it may be the cause, of the death-process; at all events it is the invariable concomitant of the latter — a fact apparently signifying that the living condition is incompatible with more than a temporary loss of impermeability.

The dependence of certain fundamental life processes (as growth, which is a manifestation of osmotic energy) on the impermeability of the plasma-membrane is more clearly apparent in plants than in animals. The proof that a similar impermeability characterizes animal cells is due mainly to the use of the plasmolytic method, which was first accurately applied to plants by de Vries, and has later been extended to animal cells by Hamburger, Overton, Koeppe and others. The principle of the method is well known; the turgor-pressure of plant cells, due to the osmotic pressure of the cell-contents, may be compensated by the application of an external osmotic pressure, *provided* that the boundary surface of the protoplast is impermeable also to the external solute; over-compensation, with compression of water from the cell and a shrinkage of the protoplast from the cellulose cell-wall, results if the external pressure distinctly exceeds that of the cell-contents; under-compensation leads to absorption of water; while with equality of external and internal osmotic pressures no osmotic change is seen (cases of hypertonic, hypotonic and isotonic solutions respectively). A means is thus afforded of testing the permeability of the plasma-membrane to different substances. If, using a moderately hypertonic solution of a given substance, a loss of water results which remains permanent during the period of immersion in the solution, impermeability relatively to that substance is indicated (the case of sugars, polyhydric alcohols, neutral salts). If the cell loses water only temporarily, afterwards regaining its original proportions, or even swelling still further, *gradual* entrance of the substance into the cell is indicated; such effects usually appear with solutions of urea, glycerine, or glycol, to which most cells are slowly permeable. If no plasmolysis results, even with strongly hypertonic solutions, a free permeability to the dissolved substance must exist; hence

such solutions typically induce swelling of cells; many organic substances, particularly fat-solvents, belong to this group. Another method of testing permeability has been employed by Hedin, which has the advantage of permitting of quantitative application. A known quantity of the substance to be tested is added to a given volume of a somewhat dense suspension of the cells studied (chiefly blood corpuscles). After an interval the mixture is centrifuged and the partition of the added substance between cells and suspension-medium may then be determined cryoscopically. The results gained by these two methods agree closely. The permeability of animal cells has been studied in considerable detail by various modifications of these methods. Overton in particular has compared the respective permeabilities of animal and plant cells and has shown that essentially the same relations hold for both. The conditions are highly constant and characteristic. All the cells examined have proved impermeable to sugars, polyhydric alcohols (erythrite and higher), amino-acids (glycocoll, alanin, leucin, asparagin, taurin), and most neutral salts of alkali and alkali earth metals; difficultly permeable to dihydric alcohols, urea and glycerine; and freely permeable to monohydric alcohols, ethers, esters, aldehydes, normal and substituted hydrocarbons — in general to such substances as exhibit in common the property of dissolving fats or of being dissolved in them. In applying such methods it must be borne in mind that many substances, including particularly the last named group, alter the normal permeability of the plasma-membrane; allowance has therefore to be made for changes in the normal permeability due to the direct action of the substances investigated. Most of the substances of the first and second of the above groups have, however, relatively slight action of this kind; and impermeability or difficult permeability to these substances appears to be a very general if not a universal rule for both plant and animal cells.

Impermeability to sugars, polyhydric alcohols, amino-acids, neutral salts, combined with ready permeability to those various organic substances which have in common fat-dissolving or lipolytic properties, appears thus to be a fundamental characteristic of living cells. The view that the surface layer of protoplasm is fatty

in nature, early expressed by Quincke and others, has thus been confirmed by a study of the permeability, since fat-solvents will readily enter and traverse a layer consisting largely of fatty substances. Overton has shown that the permeability of cells is in general such as would be expected if the plasma-membrane consisted largely of lecithin and cholesterin, the chief members of the so-called lipoid group of substances, which appear to be constant constituents of protoplasm. He found that those dyes which readily enter cells (*intra vitam* dyes) are very generally soluble in lipoid mixtures or in organic solvents containing lipoids — even if insoluble in triolein and other simple glycerides — while the group of non-vital dyes, particularly the sulphonic acid derivatives, are insoluble in such solvents.[1] A relation between lipoid-solubility and ability to enter cells thus exists for dyes, according to Overton. The rule that such solubility implies ready penetration of a dye into a cell and *vice versa* appears, however, not to be without exceptions;[2] the matter is complicated by the readiness with which the normal permeability may be modified by the very presence of the substance whose penetration is in question. There are, however, other facts which indicate that the distinctive vital permeability of cells is intimately related to the presence of these substances. Chief among these are the peculiar relations of lecithin and cholesterin to hæmolysis, a change which seems to depend primarily on alteration of the permeability of the surface layer of the erythrocytes; the action of various hæmolytic substances (saponin, agaricin, solanin, cobra poison, tetanolysin) is furthered or checked in a characteristic manner by the addition of lipoids to the blood serum. Again, the observations of Pascucci[3] in Hofmeister's laboratory on the permeability of artificial membranes impregnated with lipoids, and on the composition of the stroma of blood corpuscles, afford strong support to the view that lipoids play an essential part in the formation of the plasma-membrane. Naturally the latter is not to be regarded as a simple layer of lipoids; it appears rather to be a surface film of highly complex composi-

[1] Overton, "Jahrbücher für wissenschaftliche Botanik," 34, 1900, p. 669.

[2] Cf. the critique of Brailsford Robertson, *Journal of Biological Chemistry*, 1908, vol. 4, p. 1. Robertson nevertheless regards the surface film as partly composed of lipoids, although he ascribes more importance to the surface film of modified protein.

[3] Pascucci, *Hofmeister's Beiträge*, 1905, 6, pp. 543, 552.

tion, probably consisting of a mixture of substances, chiefly protein but largely lipoid, that have collected at the surface in consequence of their effect in lowering surface-tension. Numerous observations have shown that coherent films may thus be formed at the boundary surface between two fluids.[1] The phenomenon is now recognized as a special instance of the operation of a principle first defined by Willard Gibbs: conditions of equilibrium require that substances which lower the surface-tension of a solvent should collect in the surface layers in higher concentration than in the interior. Proteins and lipoids show this behavior. It must be granted, however, that the conditions which render the plasma-membrane of resting cells so peculiarly impermeable to soluble substances — provided these do not alter its chemical composition or colloidal consistency — are imperfectly understood. The facts of electrical stimulation indicate that the characteristic electrically polarized condition or physiological polarization of the plasma-membrane is a factor of fundamental importance.

During the greater part of its existence the cell thus appears almost completely shut off from osmotic or diffusive exchange with its surroundings. It is evident, however, that this impermeability to the food-materials and to the salts of protoplasm cannot exist at all times and under all conditions; such substances do enter the cell, and since such entrance implies penetration of the surface layer, the latter must under certain conditions become permeable. A specifically physiological problem is here encountered; either soluble food substances reach the interior of the cell quite otherwise than by a simple process of diffusion, or the permeability of the plasma-membrane must at certain times undergo functional alterations so as then to admit such substances. There is in fact evidence that the permeability of the plasma-membrane does increase at times, *e. g.*, under the influence of carbon dioxide, or during stimulation; and it is possible that food materials may enter chiefly or exclusively at such times. Nevertheless, the whole question as to the mode of entrance of soluble food materials into cells still presents many difficulties.

[1] Cf. Ramsden, *Zeitschrift für physikalische Chemie*, 1904, vol. 47, p. 336. Metcalf, *ibid.*, 1905, vol. 52, p. 1. Literature and review in this paper.

The process probably requires the performance of work on the part of cells, just as does the separation of a secretion by a gland. The kidney performs osmotic work in concentrating its secretion, and the cells of the intestinal mucosa absorb many substances against concentration gradients, a capability also implying performance of work. The rapidity with which salts and food-materials are absorbed seems indeed incompatible with the existence of an impermeable plasma-membrane; yet whenever resting cells are tested with regard to their physical permeability the above unequivocal results appear. Evidently the decisive factors in absorption as also in secretion are largely independent of physical diffusion.[1]

Whatever the actual case may be — and the problem remains unsolved for the present — there is no doubt that impermeability to the diffusion of many dissolved substances of low molecular weight is a highly distinctive and even necessary characteristic of living cells. The following considerations will make this clear. Substances of relatively low molecular weight and high diffusibility form an important part of the living protoplasmic complex. In the specific metabolism of any animal the protein and carbohydrate food materials are split respectively to amino-acids and sugars, both highly diffusible substances; and many other diffusible products important in metabolism are formed by oxidation or hydrolysis. These substances must not be lost from the cell. It is plain that any specific organism must exhibit a constant and specific metabolism — is indeed the product or the manifestation of this. Now the existence of specific metabolic processes in any cell requires the presence of many interacting substances in proportions that must not vary widely from a constant mean; in other words, constancy in the character of its metabolic processes is essential to the specificity of a particular cell. Its protoplasm may be regarded as a mixture of diverse yet constantly present substances in an approximate chemical equilibrium of a highly complex order. Any such constancy of composition, implying constancy in the conditions of equilibrium, would be impossible in a system not very completely isolated from its surroundings. In

[1] Cf. Asher, *Biochemische Zeitschrift*, 1908, XIV., p. 1, "Untersuchungen über die physiologische Permeabilität der Zellen."

the cell this isolation is due to the presence of an impermeable surface film. A marked degree of impermeability to the great majority of its diffusible constituents is thus indispensable to the continued existence of a highly complex heterogeneous system like the cell. To certain substances like oxygen and carbon dioxide the plasma-membrane appears quite freely permeable, though even in the case of these substances the permeability seems to differ greatly at different times.[1] In general the conclusion seems justified that the presence of this impermeable boundary is an indispensable condition of the most fundamental chemical characteristics of living matter. We may say indeed that just as the high development of chemistry would have been impossible without the use of vessels in which the interacting substances could be confined and isolated from the surroundings, so the development in phylogenesis of the complex chemical specificity of living organisms has depended on the isolation of the protoplasmic chemical system from its surroundings by a physically impermeable boundary. The impermeability of the plasma membrane thus appears not as a merely incidental character of living cells, but as a primary condition both of their development and of their continued existence.

The non-permeability of cells to many electrolytes is an especially significant characteristic. Neutral salts of the alkali and alkali earth metals are almost invariable constituents of protoplasm; yet the plasma-membrane, as the investigations of Overton and others have shown, is impermeable or difficultly permeable to many if not all of these. This implies impermeability to the undissociated molecules and to one or other or both of the two classes of ions resulting from dissociation. If a membrane is partially permeable to both ions of an electrolyte the chances are that it will be unequally permeable to these; and the case is conceivable that it should be freely permeable to one ion but not to the other. Ostwald[2] in 1890 first directed attention to this possibility; such a membrane, if interposed between two unequally concentrated solutions of the electrolyte, would then be the seat of an electrical potential difference, since there would be a sepa-

[1] The permeability to fat-solvents is an incidental, not a vital, peculiarity, since these substances are not normally present in protoplasm or its surroundings.

[2] *Zeitschrift für physikalische Chemie*, 6, 1890, p. 71.

ration of electricities due to the passage of a certain small proportion of ions of one sign through the membrane; this would continue until the electrostatic tension thus arising balanced the osmotic pressure difference between the ions on opposite sides of the membrane, when the ionic transfer would cease. A certain potential difference — constant (at a given temperature) with unchanged permeability of the membrane and concentrations of the electrolyte — would then exist between opposite surfaces of the membrane. The presence of membranes having such a differential permeability in reference to the anions and cations of the tissues might, Ostwald pointed out, be the source of the electrical phenomena of living organisms; and this suggestion has been further developed by Bernstein, followed by Brünings, Hoeber and others. The theory based on this interpretation — the so-called "membrane theory" of the origin of the bio-electric currents — appears to-day as the most adequate explanation of these complex and puzzling phenomena. Its merit consists mainly in the introduction of a third variable — in addition to character and concentration of the ions at the boundary surface[1] — namely, the ionic permeability of the membrane itself. This is subject to more or less sudden alteration either in the direction of increase or decrease; and the sudden and pronounced electrical changes accompanying stimulation and inhibition, as well as the remarkably high potential differences found in some instances (electric fishes), become readily intelligible on this theory.

The outer uninjured surface of a resting cell — *e. g.*, a muscle cell[2] — always proves positive relatively to the interior whenever the latter is exposed by any kind of injury or by chemical or other alteration of the surface. The membrane theory therefore assumes that the plasma-membrane is freely permeable during rest to the cations of a certain electrolyte or electrolytes contained within the cell, but not to its anions. This condition may, however, undergo temporary alteration, as during stimulation, when there is invariably a fall in the potential difference between exte-

[1] Assuming constant temperature.

[2] Of course observation cannot be directly made on single cells — unless on certain egg-cells — a possibility exemplified by Miss Hyde's investigations cited below. The above statement assumes that what is observed for parallel bundles of cells, *e. g.*, such a muscle as the frog's sartorius, is true for single components of the bundle.

rior and interior of the cell, such as would result from an increased permeability to anions. During life such potential changes occur only temporarily; they are an apparently inseparable accompaniment of any form of stimulation. On the death of the cell, however, there follows a marked and permanent increase in the general permeability, and this change is always associated with a permanent fall in the potential difference between surface and interior. That the normal potential difference observed during life is correlated with the normal impermeability of the plasma-membrane thus appears highly probable. The plasma-membrane is hence to be regarded as the seat, during life, of a permanent electrical polarization which is diminished during stimulation, and disappears or is greatly diminished at death, when the vital semi-permeability is lost. The cation to whose penetration the external positivity is due was at first supposed to be potassium, which is present in relatively large proportion in the interior of vertebrate muscle cells; but this is now known not to be the case. Were it so stimulation would involve a loss of potassium salts from the cell, of which there is no evidence; moreover Hoeber has shown that increasing the external concentration of potassium ions does not reverse the direction of the demarcation current. The indications point strongly to the rapid and highly penetrating hydrogen ion as the source of the outer positive potential of cells; and since acids, particularly carbonic acid, are known to be formed in metabolism and to leave the cell during stimulation, there exists in fact within the cell a constant source of hydrogen ions to account for the characteristic surface polarization. That such a system should show a surface polarization of the above kind is, in fact, not surprising. The depolarization associated with stimulation and with the death process implies the loss of the polarizing electrolyte from the cell; it has long been known that carbonic and possibly other acids are evolved at such times in greatly increased quantity.

Proofs that the surface layer of cells is unequally permeable to the anions and cations of many electrolytes have been furnished by the investigations of Hamburger, Koeppe, and Höber.[1]

[1] An account of these investigations is given in Höber's "Physikalische Chemie der Zelle," pp. 303 *seq.*

These have yielded the result that under the influence of carbonic or other weak acids (*i. e.*, of H-ions in low concentration) blood corpuscles give evidence — by showing an exchange of anions with the surroundings or by characteristic behavior in electrical convection — of becoming much more freely permeable to various anions (Cl, Br, SO_4, HCO_3, NO_3) than before, while the permeability to cations appears unchanged. This increase in permeability to anions is readily reversible on removing the carbon dioxide. Proof is thus afforded that the permeability of cells to ions is a variable quantity, which may undergo increase or decrease according to circumstances. The theoretical importance of a demonstration of reversible changes in ionic permeability is evident when we consider the essential rôle which, on the membrane theory, such changes play in stimulation.

The permeability of the living cell is thus not a constant factor, but one subject to alteration under a variety of conditions. The reversible changes induced by stimulating or inhibiting agencies, whose respective action appears to consist mainly in increasing or decreasing the normal permeability, are of particular physiological interest. Alterations of permeability are also induced by a large class of foreign substances which act directly upon the plasma-membrane. Such are in general: (1) The class of lipolytic substances or organic fat-solvents; these directly affect the lipoids of the plasma-membrane; if present in sufficient concentration they produce marked increase in the permeability of the surface layer of cells. The effect is conspicuous in pigment-containing cells (erythrocytes, egg-cells, etc.); the pigment leaves the cells and colors the solution (hæmolytic action of fat-solvents). Increase of permeability sufficient to allow the exit of colloid substances is usually irreversible and destructive to the cell. (2) Various electrolytes; these affect primarily the aggregation-state of the colloids. Neutral salts of alkali and alkali earth metals produce largely *reversible* changes in permeability; with salts of heavy metals, and with acids and alkalis above low concentrations the effects are irreversible, hence (in part at least) the greater toxicity of this class of electrolytes; alkalis may have also a saponifying action. (3) A large and miscellaneous class of poisonous substances — cytotoxins, hæmolysins, alkaloids and gluco-

sides of various kinds (saponin, solanin, agaricin, quillain, etc.); these increase the permeability of blood corpuscles, liberating hæmoglobin and eventually destroying the cell. Such substances alter the plasma-membrane partly by their action on the lipoids (lipoid-soluble alkaloids), partly by some specific action on the proteins, as apparently in the case of foreign blood sera. (4) Photodynamic substances (eosin, fluorescein, chlorophyll,[1] etc.); these also appear to act in the light primarily on the plasma-membrane, increasing its permeability. Tappeiner's observations[2] indicate clearly that eosin solutions first effect the peripheral layers of the cell. Paramæcia exposed to solutions of the dye in the dark, washed free, and then exposed to the light are but little affected; blood corpuscles show a certain difference in behavior and allow some entrance of the dye in the dark, but hæmolysis is much more rapid if the corpuscles are illuminated while in the solution, than if, after equally prolonged treatment in the dark, they are transferred to an eosin-free medium and then illuminated: *i. e.*, the effect is largely if not exclusively dependent on a surface action. Harzbecker and Jodlbauer[3] find the same general result. Microscopical observation also indicates that the initial stage of the destructive action consists in a disturbance of the osmotic equilibrium between corpuscle and medium, such as would result if the semi-permeability of the plasma-membrane were abolished. The corpuscles first swell, indicating entrance of water; this occurs before any perceptible entrance of the dye. P. v. Baumgarten[4] finds the same initial change in the action of hæmolytic substances, and has referred hæmolysis primarily to a loss of osmotic equilibrium consequent on alteration of the surface layer. This view is also upheld by Hamburger.[5] The increase in permeability, in addition to destroying the osmotic equilibrium, will naturally further the entrance of the toxic substance and the action will then affect the entire cell. In general toxic action must be regarded as depending in many cases primarily on an alteration, particularly an *increase*, in the normal permeability of the cells.

[1] Hausmann, *Biochemische Zeitschrift*, 1909, XVI., p. 294.

[2] Tappeiner, *Biochemische Zeitschrift*, 1908, XII., p. 290; *ibid.*, XIII., p. 1.

[3] Harzbecker u. Jodlbauer, *Biochemische Zeitschrift*, 1908, XII., p. 306.

[4] P. v. Baumgarten, *ibid.*, 1908, XI., p. 21.

[5] Hamburger, *loc. cit.*, vol. 3, p. 360.

Hence the hæmolytic action of many toxins: the injury to the plasma-membranes is naturally not confined to the blood corpuscles — the effect is merely most plainly evident in these cells; but all or most of the cells of the organism are to be regarded as similarly affected.

The above substances produce irreversible or toxic changes in cells by influencing through their chemical or solvent or coagulative action the permeability of the plasma-membrane. Essentially similar effects may result in many cases from moderate rise of temperature. Thus exposure of frog's muscle to temperatures approaching 40° for a short time produces the typical phenomenon known as heat-rigor, in which the muscle shortens and thickens while its substance undergoes complete coagulation and acquires a pronounced acid reaction. A closely similar change results when a muscle is immersed in a saturated solution of a lipoid-solvent like chloroform in an indifferent medium (*e. g.*, Ringer's solution). In both cases the changes in the muscle are associated with a loss of semi-permeability and of electrical polarization; and they are almost undoubtedly direct consequences of this since neither the moderate rise of temperature nor the presence of chloroform has by itself any such coagulative effect on the colloids composing the muscle substance; while loss of semi-permeability, with consequent disturbance of chemical equilibrium and production of acids, may readily produce just such effects.[1] Why such moderate heat should so profoundly alter the properties of the plasma-membrane is not evident at first sight; possibly the colloidal aggregation-state is the condition immediately influenced by the change of temperature; possibly the primary effect is a chemical one.

The above are instances of artificially produced or pathological changes in permeability. Normal or functional changes in this property appear to be of frequent occurrence in living organisms. Thus there is highly conclusive evidence that the general process of stimulation is dependent on a temporary and reversible increase in permeability. Very clear indications of this kind are

[1] Compare my former paper, *American Journal of Physiology*, 1908, XXII., pp. 81–83. Vernon, *Journal of Physiology*, 1899, XXIV., p. 239, and Meigs, *Amer. Journ. Physiol.*, 1909, XXIV., p. 178, also regard heat-rigor as having no direct connection with the heat coagulation of the proteins of muscle.

seen in motile plant organs. The sudden movements of Mimosa leaves, for example, result from a loss of turgor of the pulvinus cells, due to escape of cell-sap — a process which, as Sachs[1] long ago pointed out, signifies temporary increase in permeability. Since the movement is induced by the same stimuli as cause contractions in muscle-cells, and since it is accompanied by a similar electrical variation or action-current (as Burdon-Sanderson first showed), the inference seems natural that the primary change in the stimulation of animal cells is also a temporary increase in permeability. It remains therefore to inquire how such a change could lead to such seemingly disproportionate liberation of chemical and mechanical energy. I have recently discussed the relation of permeability-changes to stimulation in some detail and need not repeat *in extenso* the facts and arguments presented in these papers.[2] Briefly these are as follows: (1) Artificially-induced increase in permeability through the action of various electrolytes, lipoid-solvents, or protoplasmic poisons, produces contraction in muscle cells; (2) the death change, which is associated with an increase in permeability, produces the same effect; (3) the electrical change always associated with stimulation is of a kind indicating temporary depolarization of the plasma-membrane; (4) the loss of irritability at the height of stimulation (refractory period) is what should be expected if the plasma-membrane becomes freely permeable to ions at such times; (5) there are certain inorganic phenomena, showing remarkably close analogies to stimulation, where the effect depends on dissolution of a surface-film (pulsatile mercury and hydrogen peroxide catalysis of Bredig and his pupils); finally (6) the chemical effects of stimulation — increased production of carbon dioxide with increased acidity of the muscle substance, etc.— receive a consistent explanation on physico-chemical grounds if it is assumed that with the increase in permeability the resistance to the escape of the products of oxidation — particularly carbon dioxide — and hence also to the progress of the oxidative energy-yielding reaction is suddenly diminished; the velocity of this reaction hence under-

[1] Cf. Sachs' "Lectures on the Physiology of Plants," 1882. English translation by Marshall Ward, Oxford, 1887, p. 653.

[2] *American Journal of Physiology*, 1908, XXI., p. 200; XXII., p. 75; 1909, XXIV., p. 14.

goes a corresponding sudden increase. Inhibition is to be regarded as the inverse of stimulation and as dependent on still further decrease of the normal resting permeability.

Other instances of functional increase in permeability are apparently seen in gland cells during periods of activity. Certain influences that stimulate or heighten the irritability of muscle cells, as the action of pure solutions of many sodium salts, also, according to Fischer's and J. B. MacCallum's researches in Jacques Loeb's laboratory,[1] increase the permeability of the kidney tubules and of the intestinal epithelium; the effect is checked or counteracted by the presence of calcium salts, as also in the analogous case of muscular twitchings; and according to MacCallum hæmolytic substances — substances that increase the permeability of blood corpuscles — very generally exhibit a diuretic action, *i. e.*, increase the permeability of the kidney cells. The fact that stimulation of a gland through its nerve, as well as of a muscle, has as one of its consequences a characteristic electrical variation also indicates an increase in ionic permeability during stimulation. On the other hand, the recent results of Asher[2] with the salivary gland and the liver do not altogether bear out MacCallum's interpretation, but indicate that with such cells the specific selective power or "physiological permeability" is largely independent of externally induced changes in physical permeability. The selective action of gland cells appears indeed to be their distinctive property, and this must depend on work performed by the gland cell. Nevertheless alterations in physical permeability in all probability play an important rôle in glandular activity, as the conditions in the kidney indicate, although in the case of glands with highly developed selective properties, as the mammary gland or the liver, this factor may appear of subordinate importance.

In a rhythmically automatic tissue like cardiac muscle the phenomena of the action-current indicate on the above theory a regular alternation of periods of increased and decreased permeability corresponding respectively to the contraction and the relaxation phases of the beat. Similar rhythmical changes in permea-

[1] University of California Publications, Physiology, 1904–5, Vols. I., II.

[2] Asher, *loc. cit.*

bility must be assumed to exist in other tissues that show a markedly periodic activity, *e. g.*, automatic nerve centers like the respiratory center, or contractile structures like cilia and contractile vacuoles. The case of cell-division, as seen for example in the cleavage of an egg-cell, is another instance of a rhythmical process which, reasoning from the above considerations, one might expect to find associated with, and perhaps conditioned by, a periodically recurring increase of permeability. It is in fact noteworthy that conditions which produce increased permeability in other cells — as shown by their stimulating or hæmolytic action — very generally initiate cell-division in unfertilized eggs; such are the action of hypertonic solutions, of specific chemical substances (weak acids, potassium salts, alkalis, various coagulative, cytolytic and lipolytic substances), of mechanical treatment, of temperature changes, and of the electric current under certain conditions.[1] Loeb, in a remarkable series of experiments during the past two years, has shown that, in addition to the class of lipoid solvents, such substances as saponin, solanin, bile-salts, soaps, foreign blood sera — in general substances which, as shown by their hæmolytic action, increase the permeability of the surface layers of cells — may initiate cell-division in unfertilized ova. The surface-layer of the egg first undergoes alteration with the separation of a thin film — probably a haptogen membrane consisting mainly of protein material — the fertilization-membrane. Any condition that produces this characteristic change may lead to cell-division. The process by which the membrane is formed is regarded by Loeb as of the same nature as the cytolytic process which follows more prolonged action of the membrane-forming solutions.[2] Cytolysis, however, may be simply a consequence of loss of osmotic equilibrium, as above seen. The significance of the fertilization-membrane has been the subject of much discussion. The mere separation of a thin film from the egg-surface is probably an accessory or accidental feature of the essential change involved; it seems clear, however, that one important effect must result from the removal of such a layer of material from the

[1] Cf. the recent experiments of Delage, *Archives de zoologie expérimentale et générale*, Sér. 4, T. 9, 1908, notes et revue, p. xxx.

[2] Cf. J. Loeb, *Biochemische Zeitschrift*, 15, 1909, p. 269.

plasma-membrane; the latter is, temporarily at least, *thinned* and its permeability will therefore be increased. This increase in permeability is, on the present view, the primary event in the initiation of cell-division.

Why should such increase in permeability — granting that this is the primary change induced by the above various forms of treatment — initiate so apparently complex a process as mitotic cell-division? The main factors in producing this effect are in my opinion two: first, a disturbance of chemical equilibrium due to an increase in the rate at which certain metabolic products (probably chiefly carbon dioxide) are lost from the cell; this is an effect similar to that which, on the general theory of stimulation outlined above, underlies the chemical effect of stimulation in muscle; the precise effect of such a change will of course vary from cell to cell. Second, a definitely localized increase in the general surface-tension of the cell in consequence of a loss or lowering of the electrical surface polarization.

If the entire cell be regarded as a drop of fluid with a distinct tension at its boundary surface, it is apparent that a relative increase in surface-tension over the general surface of the two hemispheres combined with a relative decrease at the equator would result in a drawing of the material toward the two former areas and away from the equatorial region: a division of the drop into two might thus result. Robertson has recently drawn attention to this possibility, and by a simple but ingenious experiment has shown that a floating oil droplet may be made to divide symmetrically into two by locally lowering the surface-tension along its equator by means of a thread containing alkali or soap solution.[1]

[1] Robertson, *Archiv für Entwicklungsmechanik*, 27, 1909, p. 29.

It would seem that the effect of locally increasing the surface-tension would vary with the mobility or fluidity of the substance composing the system. If the surface layer possessed a high degree of viscosity approaching solidity an increase in surface-tension over an equatorial band-like area encircling the cell might produce constriction — just as would occur, *e. g.*, in an inflated rubber ball if the tension were sufficiently increased about its equator. This was Bütschli's supposition, which was later favored by Erlanger and Conklin; and by myself in an early paper. Dividing cells, however, act very plainly like fluid systems (at least in many cases, *e. g.*, starfish eggs), and there are many theoretical grounds for preferring the above point of view (see below). If the egg behaves like a drop of fluid the distribution of surface-tension assumed above would produce the observed change of form.

It can be shown by an application of the Lippmann-Helmholtz theory, connecting variations of surface-tension with changes in the potential difference at the boundary surface between two phases,[1] that increase in the ionic permeability of the plasma-membrane must result in an increase in the surface-tension of the cell. The original theory of Helmholtz[2] — that the tension between (*e. g.*) mercury and the adjoining solution is maximal with zero potential difference, and diminishes as the potential difference between the phases increases — in consequence of the static charge effects at the boundary — has required modification of recent years, since the specific effect of the ions at the boundary appears, apart from the potential difference, to modify the surface-tension.[3] Gouy's[4] extensive studies have shown that various substances, including many non-electrolytes, may modify the surface-tension of mercury. The observed surface-tension in any special case is thus dependent on (1) the specific nature of the adjoining surfaces; (2) the presence of foreign substances at the boundary, and (3) the potential difference across the boundary. In view of the sharply defined discontinuity between (*e. g.*) an egg-cell and its medium the two may be regarded as separate phases,[5] and the tension of the cell surface may be justifiably regarded as subject to the same laws as that of the mercury surface. The nature of the substances at the boundary surface and the potential difference across this surface are thus the factors to be considered. Although both are imperfectly known, certain facts seem well established. The surface film of cells contains fatty substances and proteins, both of which have marked effects in lowering the surface-tension of water; they will thus, in accordance with Gibbs' principle, tend to collect at the boundary, and the characteristics of the plasma-membrane are no doubt largely due to this

[1] Lippmann, "Relations entre les phénomènes electriques et capillaires," *Annales de chimie et de physique*, 5me Série, T. 5, 1875, p. 494.

[2] Helmholtz, "Gesammelte Abhandlungen," Bd. I., 1882, p. 925.

[3] Cf. S. W. J. Smith, *Philosophical Transactions*, Series A, 1900, 193, p. 47. Also the papers of Warburg, G. Meyer, and Paschen.

[4] Gouy, *Annales de chimie et de physique* (7), vol. 29, p. 145; (8), vol. 8, p. 291, and vol. 9, p. 75.

[5] The criterion for regarding two contiguous systems as distinct phases is essentially that they shall be bounded by a definite surface of discontinuity exhibiting characteristic energy relations (surface tension, etc.).

tendency. The presence of such substances implies that the surface-tension of cells is at least much lower than that of pure water in contact with air; it will be still further lowered in consequence of the electrical surface polarization. The surface-tension of protoplasm is thus undoubtedly very low, and apparently under circumstances may become even negative (*e. g.*, formation of pseudopodia); the low value of this tension is shown by the readiness with which minute portions of protoplasm — amœbæ, egg-cells, leucocytes, etc. — despite the large ratio of surface to volume, undergo flowing and spreading movements. Electrical stimulation produces rounding of amœboid cells — a result to be expected if the stimulus has a depolarizing effect with consequent increase of tension; cold produces a similar change of form — a consequence, possibly, of the characteristic negative temperature-coefficient of surface-tension.

A part of the low surface-tension of cells is to be ascribed to the existence of an electrical surface polarization. The evidence that this condition characterizes all living cells, while largely indirect in the nature of the case, must on the whole be regarded as strong; wherever a directly electrical test is practicable a potential difference between exterior and interior of living cells may be shown to exist, with outer surface positive; on stimulation, death or injury this potential difference undergoes a decrease.

The explanation why increase of ionic permeability produces partial or complete depolarization or fall in the potential difference between exterior and interior of the cell is briefly as follows. It is assumed that the plasma-membrane represents a partition freely permeable to the cations (probably hydrogen ions) of certain intracellular electrolytes (chiefly carbonic acid), but impermeable or difficultly permeable to the anions (assumed to be mainly HCO_3^- and $CO_3^=$) and to the undissociated molecules. This means that the velocity of the cation is practically unmodified by the membrane while that of the anion is greatly reduced. Under these conditions there will be a marked difference in the concentrations of the electrolyte on opposite sides of the membrane; the potential difference between the two solutions will be that observed between unequally concentrated adjoining solutions of an electrolyte with ions of unequal velocities. This potential difference E

according to Nernst's formula [1] may be estimated from the following values:

$$E = \frac{u - v}{u + v} \frac{RT}{q} ln \frac{c_2}{c_1}.$$

Generally, therefore, the greater the difference between the two ionic velocities the greater the potential difference across the boundary; hence this will be maximal with complete impermeability to the anion, since then its velocity is reduced to zero at the membrane and $u - v/u + v$ becomes unity. Normally, however, there is probably a certain permeability to the anion, since carbonic acid is continually evolved from living cells, although slowly in a state of rest; the potential difference in resting cells may thus be supposed to approach but not to reach the maximum. If then the permeability undergoes an increase so that anions may penetrate the membrane with considerable freeness, *i. e.*, with increased velocity, the value of $u - v/u + v$ and also of c_2/c_1 will decrease; the result will be a fall of the potential difference across the surface. The extent of this fall will obviously depend on the degree of the increase in permeability and may show a considerable range of variability.

With decrease in the potential difference the surface-tension must undergo a corresponding increase; this increase in surface-tension is, on the present theory, the main condition of the form change in cell-division. Other changes of form may naturally be similarly conditioned; irregular form changes are frequent in unfertilized eggs in which division is induced by the artificial parthogenetic methods; regular cleavage would require a symmetrical distribution of the areas of increased permeability with reference to the future cleavage plane.

In agreement with the above view of the nature of the chemical effects of stimulation, the initiation of the characteristic series of chemical processes in cell-division is to be referred to a disturbance of chemical equilibrium in consequence of the more rapid loss of certain materials, perhaps simply carbon dioxide,

[1] E is potential difference in volts, u velocity of cation, v that of anion, R gas constant, T absolute temperature, q quantity of electricity (coulombs) carried by a monovalent gram-ion, ln natural logarithm, c_1 and c_2 concentrations of the dissociated part of the electrolyte on opposite sides of the boundary surface. The ions are assumed to be monovalent in this form of the formula.

through the now permeable plasma-membrane. According to Lyon's[1] observations the carbon dioxide production by the dividing egg exhibits a rhythmical increase and decrease running parallel with the rhythm of cell-division; the periods of increased carbon dioxide production probably correspond to periods of increased permeability.

Such changes ought, on the membrane theory, to be accompanied by changes in the state of electrical surface polarization. Experimental proof of the existence of such changes is difficult but probably not altogether impracticable. Miss Hyde has attempted — using the capillary electrometer, with one electrode on the blastodisc of dividing fundulus eggs, the other on the opposite pole, — to determine if, during cell division, any potential difference makes its appearance between the dividing portion of the egg and its unaltered general surface.[2] While her results are not altogether unequivocal, they do seem to indicate that at or about the time of cleavage the outer surface of the blastodisc becomes negative relatively to the general outer surface of the egg — *i. e.*, undergoes a change of potential similar to that which occurs during stimulation, indicating increased permeability. These important and interesting experiments should be repeated and confirmed. There are in fact various indications that during cell-division the potential difference between the exterior and interior of the cell undergoes marked alteration. The disposition of the colloidal material of the cell at this time in the characteristic radiations is strong confirmatory evidence; this phenomenon plainly suggests the polarization of colloidal particles in a strong electrical field. It is a corollary of the above theory that with the appearance of an increased permeability — implying depolarization of the surface layer — the peripheral regions of the protoplasm must become — for a time at least, until the potentials are equalized — *positive* relatively to the interior. If the voltage of the surface potential change is comparable to that of the action-current of muscle, *i. e.*, *ca.* 0.05–0.08 volt (as is highly probable), a potential gradient of considerable steepness will exist temporarily, at the time of increased permeability, between the

[1] E. P. Lyon, *American Journal of Physiology*, 1904, XI., p. 52.

[2] I. H. Hyde, *ibid.*, 1904, XII., p. 241.

peripheral and the central regions of the protoplasm; this may be amply sufficient to account for the striking change in the configuration of the cytoplasmic colloids.[1] The peripheral layer would become positive relatively to the central region under the conditions existing in such a system as a cell with a surface polarization of the kind indicated. If we can draw safe inferences from the cytological facts the astral centers do in fact show the properties of negative regions.[2] I hope however to consider the physiology of cell-division from the above point of view more fully in a later paper.[3]

[1] It may be desirable to give here the estimate on which this statement is based. If we assume that the surface potential difference due to the physiological polarization has the same value as the potential difference of the demarcation current of muscle (*ca.* 0.08 volt) and that the fall of potential or negative variation during cleavage is equal to that of the action current (*ca.* 0.05 volt), we infer that in the resting cell there is a potential difference between exterior and interior equal to *ca.* 0.08 volt. This gradient, being due mainly to the unequal permeability of the plasma-membrane to the two ions, exists almost entirely between the inner and outer surfaces of the plasma-membrane; the interior of the cell (cytoplasm) would thus have an almost uniform negative potential of about this value with little if any fall between its central and peripheral regions. If now, in consequence of a sudden increase in permeability, the potential difference across the plasma-membrane decreases by 0.05 volt, a gradient will temporarily exist between peripheral and central regions of the protoplasm equal to this value, the most negative region being that most remote from the periphery. A gradient of 0.05 volt between the center and the periphery of a sea-urchin egg, diameter 0.072 mm., is a gradient of 0.05 volt in 0.036 mm., *i. e.*, 1 volt in 0.072 cm. or about 14 volts per centimeter. The assumed potential differences may be too high, though they are typical of the directly observed values in muscle cells. The change in permeability is also probably rather gradual than sudden. At least these considerations show that a strong electrical field may arise between central and peripheral regions of the cell under the conditions assumed. Of course no such potential difference could be *maintained* without the performance of electrical work on the part of the cell (assuming the free movements of ions within the cytoplasm); but its existence for only a few seconds might well produce the observed effects. This note, however, aims merely at justifying the above statement in the text, not at a complete discussion.

[2] Cf. my paper in *American Journal of Physiology*, 1905, XV., p. 46.

[3] In my first paper dealing with the theory of cell-division (BIOLOGICAL BULLETIN, 4, 1903, p. 175) the system of astral radiations was recognized as evidence of an electrical potential difference between peripheral and central regions of the dividing cell, and the form change was ascribed to a capillary electric change of surface-tension. The tentative hypothesis then presented to account for the general course of the process was, however, quite different from that outlined above, and, with the exception of certain details, will have to be abandoned.